FROM-Gen-Probe Inc.

REPLY

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

Amendments to the Claims

1. (Currently Amended) A hybridization assay probe comprising an oligonucleotide a target binding region from 18 to 35 bases in length that which hybridizes to a target sequence present in target nucleic acid derived from a Cryptosporidium organism organisms in a test sample under stringent conditions to form a probe:target hybrid stable for detection, said oligonucleotide having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in the target sequence, wherein the said target sequence is being selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, wherein said probe does not comprise a base region in addition to said target binding region that is capable of stably binding to said target nucleic acid under said conditions, and wherein said probe does not hybridize to nucleic acid derived from a non-Cryptosporidium organism in the said test sample to form a probe:non-target hybrid stable for detection under the stringent said conditions.

Claims 2-6 (Canceled)

- 7. (Currently Amended) The probe of claim 1, wherein said probe contains <u>at</u> least two base sequences which regions that hybridize to each other when said probe is not hybridized to the said target sequence under the stringent said conditions.
- 8. (Currently Amended) The probe of claim 1, wherein said probe comprises one or more at least one base sequences which region that do does not stably hybridize to nucleic acid derived from Cryptosporidium organisms, or to mucleic acid derived from a non-target organism present in the test sample, under the stringent said conditions.

9. Canceled

Page 2 of 37

- 10. (Original) The probe of claim 1 further comprising a detectable label.
- 11. (Original) The probe of claim + 2 further comprising a group of interacting labels.
- 12. (Original) The probe of claim 11, wherein said interacting labels include a luminescent label and a quencher label.
- 13. (Currently Amended) The probe of claim 1, wherein said oligonacleotide target binding region includes at least one ribonucleotide modified to include a 2'-O-methyl substitution to the ribofuranosyl moiety.
- 14. (Currently Amended) The probe of claim 1, wherein a pseudo peptide backbone joins at least a portion of the bases of said oligonucleotide target binding region.
- 15. (Currently Amended) The probe of claim 1, wherein the stringent said conditions comprise 50 mM succinic acid, 1% (w/v) LLS, 7.5 mM aldrithiol-2, 0.6 M LiCl, 115 mM LiOH, 10 mM EDTA, 10 mM EGTA, 1.5% (v/v) ethyl alcohol (absolute), pH to 4.7, and a test sample temperature of about 60°C.
- 16. (Currently Amended) A hybridization assay The probe of claim 1, comprising an oligonucleotide which hybridizes to a target sequence present in nucleic acid derived from a Cryptosporidium organism in a test sample under stringent conditions to form a probe target hybrid stable for detection, wherein said oligonucleotide has a the base sequence which of said target binding region is at least 80% complementary to the base sequence of the said target sequence; wherein the target sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, and wherein said probe does not hybridize to nucleic acid derived

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

from a non-Cryptosporidium organism in the test sample to form a probemon-target hybrid stable for detection under the stringent conditions.

- 17. (Currently Amended) An oligonucleotide The probe of claim 1, which hybridizes to a target sequence present in nucleic acid derived from a Cryptosporidium organism in a test sample under stringent conditions to form a probe target hybrid stable for detection, wherein the base sequence of said probe is at least 80% complementary to the base sequence of the said target sequence, wherein the target sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, and wherein said probe does not hybridize to nucleic acid derived from a non-Cryptosporidium organism in the test sample to form a probe-non-target hybrid stable for detection under the stringent conditions.
- hybridizes to a target sequence present in nucleic acid derived from a Cryptosporidium organism in a test sample under stringent conditions to form a probe target hybrid stable for detection, wherein the base sequence of said probe is fully complementary to the base sequence of the said target sequence, wherein the target sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, and wherein said probe does not hybridize to nucleic acid derived from a non-Cryptosporidium organism in the test sample to form a probe non-target hybrid stable for detection under the stringent conditions.
- 19. (Currently Amended) A probe mix comprising the <u>said</u> probe of claim 1 and a first helper oligonucleotide <u>from 18 to 35 bases in length which hybridizes to having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in a target sequence, wherein the target sequence of said first helper oligonucleotide is selected from the group consisting of SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25 and SEQ ID NO:27 under said conditions.</u>

Page 4 of 37

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

20. Canceled

21. (Withdrawn) The probe mix of claim 19 further comprising a second helper oligonucleotide having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in a target sequence, wherein the target sequence of said second helper oligonucleotide is selected from the group consisting of SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26 and SEQ ID NO:28.

22. Canceled

23. (Currently Amended) An amplification primer oligonucleotide for use in amplifying a nucleic acid sequence present in target nucleic acid derived from a Cryptosporidium organisms under amplification conditions, said primer amplification oligonucleotide comprising a target binding region from 18 to 40 bases in length which hybridizes to an oligonucleotide having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in a target sequence selected from the group consisting of SEQ ID NO:48, SEQ ID NO:54, SEQ ID NO:60 and SEQ ID NO:66 under amplification conditions, wherein said amplification oligonucleotide does not comprise a base region in addition to said target binding region that is capable of stably binding to said target nucleic acid under said conditions, and wherein said primer amplification oligonucleotide optionally includes a 5' sequence which that is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase.

Claims 24-32 (Canceled)

- 33. (Currently Amended) The primer amplification oligonucleotide of claim 23, wherein said primer amplification oligonucleotide includes the said 5' sequence which is recognized by an RNA polymerase or which enhances initiation or clongation by an RNA polymerase.
- 34. (Currently Amended) The primer amplification oligonucleotide of claim 33, wherein the said 5' sequence which is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase is a T7 promoter having the base sequence of SEQ ID NO:69.
- 35. (Currently Amended) The primer amplification oligonucleotide of claim 23, wherein said primer amplification oligonucleotide contains at least two base sequences which regions that hybridize to each other when said amplification oligonucleotide is not hybridized to the said target sequence under the amplification said conditions.
- 36. (Currently Amended) The primer <u>amplification oligonucleotide</u> of claim 35 further comprising a group of interacting labels.
- 37. (Currently Amended) The primer amplification oligonucleotide of claim 36, wherein said interacting labels include a luminescent label and a quencher label.
- 38. (Currently Amended) An The amplification oligonucleotide of claim 23. wherein the base sequence of said target binding region primer for use in amplifying a nucleic acid sequence present in nucleic acid derived from a Cryptosporidium organism under amplification conditions, said primer comprising an oligonucleotide having a base sequence which is at least 80% complementary to the base sequence of a said target sequence, selected from the group consisting of SEQ ID NO:48, SEQ ID NO:54, SEQ ID NO:60 and SEQ ID NO:66, and wherein said primer

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

optionally includes a 5' sequence which is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase.

39. Canceled

- wherein the base sequence of said target binding region primer for use in amplifying a nucleic acid sequence present in nucleic acid derived from a Cryptosporidium organism under amplification conditions, wherein the base sequence of said primer is fully complementary to the base sequence of a said target sequence selected from the group consisting of SEQ ID NO:48, SEQ ID NO:54, SEQ ID NO:60 and SEQ ID NO:66, and wherein said oligonucleotide optionally includes a 5' sequence which is recognized by an RNA polymerase or which enhances initiation or clongation by an RNA polymerase.
- 41. (Currently Amended) A set of amplification primers oligonucleotides for use in amplifying a nucleic acid sequence present in target nucleic acid derived from a Cryptosporidium organism organisms under amplification conditions, said set of primers amplification oligonucleotides, wherein:

said first primer amplification oligonucleotide is said primer amplification oligonucleotide of claim 23; and

said second primer amplification oligonucleotide comprises a target binding region from 18 to 40 bases in length which hybridizes to an oligonucleotide having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in a target sequence selected from the group consisting of SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:63 and SEQ ID NO:64 under amplification conditions, wherein said second amplification oligonucleotide does not comprise a base region in addition to said target binding region that is capable of stably binding to said target

Serial No. 09/954,695 Atty, Docket No. GP116-02.UT

nucleic acid under said conditions, and wherein one or more primers said second amplification oligonucleotide of said set of primers optionally include includes a 5' sequence which that is recognized by an RNA polymerase or which that enhances initiation or elongation by an RNA polymerase.

42. Canceled

43. (Currently Amended) The primer set of <u>amplification oligonucleotides of</u> claim 41, wherein the <u>said</u> target sequence of said second <u>primer amplification oligonucleotide</u> is selected from the group consisting of SEQ ID NO:45, SEQ ID NO:51, SEQ ID NO:57 and SEQ ID NO:63.

44. Canceled

45. (Currently Amended) The primer set of amplification oligonucleotides of claim 41, wherein the <u>said</u> target sequence of said second primer <u>amplification oligonucleotide</u> is selected from the group consisting of SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58 and SEQ ID NO:64.

Claims 46-49 (Canceled)

50. (Currently Amended) A method for determining the presence of a Cryptosporidium organisms in a test sample, said method comprising the steps of: contacting the said test sample with said probe of claim 1 under stringent conditions; and

determining whether a probe:target hybrid has formed as an indication of the presence of a *Cryptosporidium* organisms organisms in the said test sample.

Page 8 of 37

and

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

- 51. (Currently Amended) A method for determining the presence of a Cryptosporidium organism organisms in a test sample, said method comprising the steps of: contacting the <u>said</u> test sample with said probe of claim 16 under stringent conditions; and
- determining whether a probe: target hybrid has formed as an indication of the presence of a Cryptosporidium organism organisms in the said test sample.
- 52. (Currently Amended) A method for determining the presence of a
 Cryptosporidium organisms organisms in a test sample, said method comprising the steps of:

 contacting the said test sample with said probe of claim 17 under stringent conditions;
 and
 determining whether a probe:target hybrid has formed as an indication of the presence

of a Cryptosporidium organism organisms in the said test sample.

53. (Currently Amended) A method for determining the presence of a Cryptosporidium organism organisms in a test sample, said method comprising the steps of: contacting the said test sample with said probe of claim 18 under stringent conditions;

determining whether a probe:target hybrid has formed as an indication of the presence of a Cryptosporidium organism organisms in the said test sample.

- 54. (Currently Amended) A method for amplifying *Cryptosporidium* nucleic acid which that may be present in a test sample, said method comprising the steps of:
- contacting the <u>said</u> test sample with said primer <u>amplification oligonucleotide</u> of claim 23 under amplification conditions; and
- amplifying a target sequence present in target nucleic acid derived from a Cryptosporidium organism which organisms that may be present in the said test sample.

Page 9 of 37

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

Claims 55-60 (Canceled)

- 61. (Currently Amended) The method of claim 54 further comprising the step of providing to said test sample a hybridization assay probe for use in determining the presence of the whether said target sequence was amplified in said amplifying step target sequence in the test sample with a hybridization assay probe.
- 62. (Currently Amended) The method of claim 61, wherein said probe comprises an oligonucleotide which a target binding region from 18 to 35 bases in length that hybridizes to the amplified said target sequence or the complement thereof under stringent conditions to form a probe:target hybrid stable for detection, said oligonucleotide having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in the amplified target sequence, wherein the amplified said target sequence or the complement thereof is being selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, wherein said probe does not comprise a base region in addition to said target binding region that is capable of stably binding to nucleic acid derived from Cryptosporidium organisms under said conditions, and wherein said probe does not hybridize to nucleic acid derived from a non-Cryptosporidium organism in the said test sample to form a probe:non-target hybrid stable for detection under the stringent said conditions.

Claims 63-70 (Canceled)

71. (Currently Amended) A method for amplifying Cryptosporidium nucleic acid which that may be present in a test sample, said method comprising the steps of:

contacting the said test sample with said primer amplification oligonucleotide of claim 38 under amplification conditions; and

Page 10 of 37

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

amplifying a target sequence present in target nucleic acid derived from a Cryptosporidium organism which organisms that may be present in the said test sample.

72. Canceled

73. (Currently Amended) A method for amplifying *Cryptosporidium* nucleic acid which that may be present in a test sample, said method comprising the steps of:

contacting the <u>said</u> test sample with said primer <u>amplification oligonucleotide</u> of claim 40 under amplification conditions; and

amplifying a target sequence present in <u>target</u> nucleic acid derived from a Cryptosporidium organism which <u>organisms that</u> may be present in the <u>said</u> test sample.

74. (Currently Amended) A kit comprising, in packaged combination, first and second oligonucleotides for use in determining the presence of a Cryptosporidium organism organisms in a test sample, each of said oligonucleotides comprising a target binding region having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in which hybridizes to a target sequence contained present in target nucleic acid derived from a Cryptosporidium organism organisms under hybridization conditions, said target binding region of said first oligonucleotide being from 18 to 35 bases in length and said target binding region of said second oligonucleotide being from 18 to 40 bases in length.

wherein: the <u>said</u> target sequence of said first oligonucleotide is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4;

the wherein said target sequence of said second oligonucleotide is selected from the group consisting of SEQ ID NO:48, SEQ ID NO:54, SEQ ID NO:60 and SEQ ID NO:66, and

wherein neither of said first and second oligonucleotides comprises a base region in addition to said target binding region that is capable of stably binding to said target nucleic acid under said conditions, and

Page 11 of 37

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

wherein said second oligonucleotide optionally includes a 5' sequence which that is recognized by an RNA polymerase or which that enhances initiation or elongation by an RNA polymerase.

Claims 75-82 (Canceled)

83. (Currently Amended) The kit of claim 74 further comprising a third oligonucleotide, wherein said third oligonucleotide has an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in said third oligonucleotide comprising a target binding region from 18 to 40 bases in length which hybridizes to a target sequence contained present in target nucleic acid derived from a Cryptosporidium organism organisms under hybridization conditions, and wherein the said target sequence of said third oligonucleotide is being selected from the group consisting of SEQ ID NO:45, SEQ ID NO:51, SEQ ID NO:57 and SEQ ID NO:63, wherein said third oligonucleotide does not comprise a base region in addition to said target binding region that is capable of stably binding to said target nucleic acid under said conditions, and wherein said third oligonucleotide optionally includes a 5' sequence that is recognized by an RNA polymerase or that enhances initiation or elongation by an RNA polymerase.

84. Canceled

85. (Currently Amended) The kit of claim 74 further comprising a third oligonucleotide, wherein said third oligonucleotide has an at least 10 contiguous base region which is at least 80% complementary to an at-least 10 contiguous base region present in said third oligonucleotide comprising a target binding region from 18 to 40 bases in length which hybridizes to a target sequence contained present in target nucleic acid derived from a Cryptosporidium organism organisms under hybridization conditions, and wherein the said target sequence of said

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

third oligonucleotide is being selected from the group consisting of SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58 and SEQ ID NO:64, wherein said third oligonucleotide does not comprise a base region in addition to said target binding region that is capable of stably binding to said target nucleic acid under said conditions, and wherein said third oligonucleotide optionally includes a 5' sequence that is recognized by an RNA polymerase or that enhances initiation or elongation by an RNA polymerase.

Claims 86 and 87 (Canceled)

88. (Currently Amended) A kit comprising, in packaged combination, first and second oligonucleotides for use in determining the presence of a Cryptosporidium organism organisms in a test sample, each of said oligonucleotides comprising a target binding region from 18 to 35 bases in length having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in that hybridizes to a target sequence contained present in target nucleic acid derived from a Cryptosporidium organism organisms under stringent conditions,

wherein: the <u>said</u> target sequence of said first oligonucleotide is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, and

the wherein said target sequence of said second oligonucleotide is selected from the group consisting of SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25 and SEQ ID NO:27.

wherein neither of said first and second oligonucleotides comprises a base region in addition to said target binding region that is capable of stably binding to said target nucleic acid under said conditions, and

wherein said first oligonucleotide does not hybridize to nucleic acid derived from a non-Cryptosporidium organism in said test sample to form a probe:non-target hybrid stable for detection under said conditions.

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

Claims 89-91 (Canceled)

- 92. (Currently Amended) The probe of claim 16.1, wherein said oligonucleotide has a the base sequence of said target binding region is fully which is 100% complementary to the base sequence of the said target sequence.
- 93. (Currently Amended) A probe mix comprising the <u>said</u> probe of claim 16 and a first helper oligonucleotide, wherein the base sequence of said first helper oligonucleotide is at least 80% complementary to the base sequence of a target sequence selected from the group consisting of SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25 and SEQ ID NO:27.
- 94. (Previously Added) The probe mix of claim 93 further comprising a second helper oligonucleotide, wherein the base sequence of said second helper oligonucleotide is at least 80% complementary to the base sequence of a target sequence selected from the group consisting of SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26 and SEQ ID NO:28.
- 95. (Currently Amended) A probe mix comprising the <u>said</u> probe of claim 17 and a first helper oligonucleotide, wherein the base sequence of said first helper oligonucleotide is at least 80% complementary to the base sequence of a target sequence selected from the group consisting of SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25 and SEQ ID NO:27.
- 96. (Previously Added) The probe mix of claim 95 further comprising a second helper oligonucleotide, wherein the base sequence of said second helper oligonucleotide is at least 80% complementary to the base sequence of a target sequence selected from the group consisting of SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26 and SEQ ID NO:28.

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

- 97. (Currently Amended) A probe mix comprising the <u>said</u> probe of claim 18 and a first helper oligonucleotide, wherein the base sequence of said first helper oligonucleotide is fully complementary to the base sequence of a target sequence selected from the group consisting of SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25 and SEQ ID NO:27.
- 98. (Previously Added) The probe mix of claim 97 further comprising a second helper oligonucleotide, wherein the base sequence of said second helper oligonucleotide is fully complementary to the base sequence of a target sequence selected from the group consisting of SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26 and SEQ ID NO:28.
- 99. (Currently Amended) The primer amplification oligonucleotide of claim 38, wherein said oligonucleotide has a the base sequence of said target binding region which is 100% is fully complementary to the base sequence of the said target sequence.
- 100. (Currently Amended) A set of amplification primers oligonucleotides for use in amplifying a nucleic acid sequence present in target nucleic acid derived from a Cryptosporidium organisms under amplification conditions, said set of primers amplification oligonucleotides including first and second primers amplification oligonucleotides, wherein:

said first primer amplification oligonucleotide is said primer amplification oligonucleotide of claim 38; and

said second primer amplification oligonucleotide comprises an oligonucleotide having a target binding region, wherein the base sequence which of said target binding region is at least 80% complementary to the base sequence of a target sequence selected from the group consisting of SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:63 and SEQ ID NO:64,

wherein said target binding region of said second amplification oligonucleotide hybridizes to said target sequence under amplification conditions.

Page 15 of 37

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

wherein said second amplification oligonucleotide does not comprise a base region in addition to said target binding region that is capable of stably binding to said target nucleic acid under said conditions, and

wherein said second amplification oligonucleotide optionally includes a 5' sequence that is recognized by an RNA polymerase or that enhances initiation or elongation by an RNA polymerase.

- 101. (Currently Amended) The primer set of amplification oligonucleotides of claim 100, wherein the <u>said</u> target sequence of said second primer <u>amplification oligonucleotide</u> is selected from the group consisting of SEQ ID NO:45, SEQ ID NO:51, SEQ ID NO:57 and SEQ ID NO:63.
- 102. (Currently Amended) The primer set of amplification oligonucleotides of claim 100, wherein the <u>said</u> target sequence of said second primer <u>amplification oligonucleotide</u> is selected from the group consisting of SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58 and SEQ ID NO:64.

Claims 103-105 (Canceled)

106. (Currently Amended) A set of amplification primers oligonucleotides for use in amplifying a nucleic acid sequence present in <u>target</u> nucleic acid derived from a *Cryptosporidium* organisms under amplification conditions, said set of primers amplification oligonucleotides including first and second primers amplification oligonucleotides, wherein:

said first primer amplification oligonucleotide is said primer amplification oligonucleotide of claim 40; and

said second primer amplification oligonucleotide comprises an oligonucleotide a target binding region, wherein the base sequence of said primer target binding region is fully

Page 16 of 37

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

complementary to the base sequence of a target sequence selected from the group consisting of SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:63 and SEQ ID NO:64.

wherein said target binding region of said second amplification oligonucleotide hybridizes to said target sequence under amplification conditions.

wherein said second amplification oligonucleotide does not comprise a base region in addition to said target binding region that is capable of stably binding to said target nucleic acid under said conditions, and

wherein said second amplification oligonucleotide optionally includes a 5' sequence that is recognized by an RNA polymerase or that enhances initiation or elongation by an RNA polymerase.

- 107. (Currently Amended) The primer set of amplification oligonucleotides of claim 106, wherein the said target sequence of said second primer amplification oligonucleotide is selected from the group consisting of SEQ ID NO:45, SEQ ID NO:51, SEQ ID NO:57 and SEQ ID NO:63.
- 108. (Currently Amended) The primer set of amplification oligonucleotides of claim 106, wherein the said target sequence of said second primer amplification oligonucleotide is selected from the group consisting of SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58 and SEQ ID NO:64.
- 109. (Currently Amended) The method of claim 51, wherein said oligonucleotide has a the base sequence which is 100% of said target binding region is fully complementary to the base sequence of the said target sequence.

- amplification primer oligonucleotide, said second primer amplification oligonucleotide comprising a target binding region from 18 to 40 bases in length that hybridizes to an oligonucleotide having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in a target sequence selected from the group consisting of SEQ ID NO:45, SEQ ID NO:51, SEQ ID NO:57 and SEQ ID NO:63 under said conditions, wherein said second amplification oligonucleotide does not comprise a base region in addition to said target binding region that is capable of stably binding to said target nucleic acid under said conditions, and wherein said second amplification oligonucleotide optionally includes a 5' sequence that is recognized by an RNA polymerase or that enhances initiation or elongation by an RNA polymerase.
- of determining the presence of the amplicon amplified target sequence in a said test sample with a hybridization assay probe, wherein said probe comprises an oligonucleotide which a target binding region from 18 to 35 bases in length that hybridizes to the a target sequence present in said amplicon amplified target sequence under stringent conditions to form a probe:target hybrid stable for detection, said oligonucleotide having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in the amplified target sequence, wherein the said amplified target sequence is being selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, wherein said probe does not comprise a base region in addition to said target binding region that is capable of stably binding to said amplicon under said conditions, and wherein said probe does not hybridize to nucleic acid derived from a non-Cryptosporidium organism in the said test sample to form a probe:non-target hybrid stable for detection under the stringent said conditions.
- 112. (Currently Amended) The method of claim 54 further comprising a second amplification primer oligonucleotide, said second primer amplification oligonucleotide comprising

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

a target binding region from 18 to 40 bases in length that hybridizes to an oligonucleotide having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in a target sequence selected from the group consisting of SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58 and SEQ ID NO:64 under said conditions, wherein said second amplification oligonucleotide does not comprise a base region in addition to said target binding region that is capable of stably binding to said target nucleic acid under said conditions, and wherein said second amplification oligonucleotide optionally includes a 5' sequence that is recognized by an RNA polymerase or that enhances initiation or elongation by an RNA polymerase.

- of determining the presence of the amplicon amplified target sequence in a said test sample with a hybridization assay probe, wherein said probe comprises are oligonucleotide which a target binding region from 18 to 35 bases in length that hybridizes to the a target sequence present in said amplicon amplified target sequence under stringent conditions to form a probe:target hybrid stable for detection, said oligonucleotide having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in the amplified target sequence, wherein the said amplified target sequence is being selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, wherein said probe does not comprise a base region in addition to said target binding region that is capable of stably binding to said amplicon under said conditions, and wherein said probe does not hybridize to nucleic acid derived from a non-Cryptosporidium organism in the said test sample to form a probe:non-target hybrid stable for detection under the stringent said conditions.
- 114. (Currently Amended) The method of claim 71 further comprising the step of determining the presence of the amplicon amplified target sequence in a said test sample with a hybridization assay probe, wherein said probe comprises an oligonucleotide which a target binding region that hybridizes to the a target sequence present in said amplicon amplified target sequence

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

under stringent conditions to form a probe:target hybrid stable for detection, wherein said oligonucleotide has a the base sequence of said target binding region which is at least 80% complementary to the base sequence of said target sequence amplified target sequence, wherein the said target amplified target sequence is being selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, wherein said probe does not comprise a base region in addition to said target binding region that is capable of stably binding to said amplicon under said conditions, and wherein said probe does not hybridize to nucleic acid derived from a non-Cryptosporidium organism in the said test sample to form a probe:non-target hybrid stable for detection under the stringent said conditions.

amplification primer oligonucleotide, said second primer comprising an oligonucleotide having a target binding region, wherein the a base sequence of said target binding region which is at least 80% complementary to a the base sequence of a target sequence present in target nucleic acid derived from Cryptosporidium organisms, said target sequence being selected from the group consisting of SEQ ID NO:45, SEQ ID NO:51, SEQ ID NO:57 and SEQ ID NO:63.

wherein said target binding region hybridizes to said target sequence under said conditions,

wherein said second amplification oligonucleotide does not comprise a base region in addition to said target binding region that is capable of stably binding to said target nucleic acid under said conditions, and

wherein said second amplification oligonucleotide optionally includes a 5' sequence that is recognized by an RNA polymerase or that enhances initiation or elongation by an RNA polymerase.

116. (Currently Amended) The method of claim 115 further comprising the step of determining the presence of the amplicon amplified target sequence in a said test sample with a

Page 20 of 37

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

hybridization assay probe, wherein said probe comprises an oligonucleotide which a target binding region that hybridizes to the a target sequence present in said amplicon amplified target sequence under stringent conditions to form a probe:target hybrid stable for detection, wherein said oligonucleotide has a the base sequence of said target binding region which is at least 80% complementary to the base sequence of said target sequence amplified target sequence, wherein the said amplified target sequence is being selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, wherein said probe does not comprise a base region in addition to said target binding region that is capable of stably binding to said amplicon under said conditions, and wherein said probe does not hybridize to nucleic acid derived from a non-Cryptosporidium organism in the said test sample to form a probe:non-target hybrid stable for detection under the stringent said conditions.

117. (Currently Amended) The method of claim 71 further comprising a second amplification primer oligonucleotide, said second primer comprising an oligonucleotide having a target binding region, wherein the a base sequence of said target binding region which is at least 80% complementary to a the base sequence of a target sequence present in target nucleic acid derived from Cryptosporidium organisms, said target sequence being selected from the group consisting of SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58 and SEQ ID NO:64.

wherein said target binding region hybridizes to said target sequence under said conditions.

wherein said second amplification oligonucleotide does not comprise a base region in addition to said target binding region that is capable of stably binding to said target nucleic acid under said conditions, and

wherein said second amplification oligonucleotide optionally includes a 5' sequence that is recognized by an RNA polymerase or that enhances initiation or elongation by an RNA polymerase.

- of determining the presence of the amplicon amplified target sequence in a said test sample with a hybridization assay probe, wherein said probe comprises an oligonucleotide which a target binding region that hybridizes to the a target sequence present in said amplified target sequence under stringent conditions to form a probe:target hybrid stable for detection, wherein said oligonucleotide has a the base sequence of said target binding region which is at least 80% complementary to the base sequence of said amplified target sequence, wherein the said amplified target sequence is being selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, wherein said probe does not comprise a base region in addition to said target binding region that is capable of stably binding to said amplicon under said conditions, and wherein said probe does not hybridize to nucleic acid derived from a non-Cryptosporidium organism in the said test sample to form a probe:non-target hybrid stable for detection under the stringent said conditions.
- 119. (Currently Amended) The method of claim 71, wherein said oligonucleotide has a the base sequence which of said target binding region is 100% fully complementary to the base sequence of the said target sequence.
- of determining the presence of the amplicon amplified target sequence in a said test sample with a hybridization assay probe, wherein said probe comprises an oligonucleotide which a target binding region that hybridizes to the a target sequence present in said amplicon amplified target sequence under stringent conditions to form a probe:target hybrid stable for detection, wherein the base sequence of said oligonucleotide target binding region has a base sequence which is 100% fully complementary to the base sequence of said amplified target sequence, wherein the said amplified target sequence is being selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, wherein said probe does not comprise a base region in addition to said

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

target binding region that is capable of stably binding to said amplicon under said conditions, and wherein said probe does not hybridize to nucleic acid derived from a non-Cryptosporidium organism in the said test sample to form a probe:non-target hybrid stable for detection under the stringent said conditions.

121. (Currently Amended) The method of claim 119 further comprising a second amplification primer oligonucleotide, said second primer comprising an oligonucleotide having a target binding region, wherein the a base sequence which of said target binding region is 100% fully complementary to a the base sequence of a target sequence present in target nucleic acid derived from Cryptosporidium organisms, said target sequence being selected from the group consisting of SEQ ID NO:45, SEQ ID NO:51, SEQ ID NO:57 and SEQ ID NO:63,

wherein said target binding region hybridizes to said target sequence under said conditions.

wherein said second amplification oligonucleotide does not comprise a base region in addition to said target binding region that is capable of stably binding to said target nucleic acid under said conditions, and

wherein said second amplification oligonucleotide optionally includes a 5' sequence that is recognized by an RNA polymerase or that enhances initiation or elongation by an RNA polymerase.

of determining the presence of the <u>amplicon</u> amplified target sequence in a test sample with a hybridization assay probe, wherein said probe comprises an oligonucleotide which a target binding region that hybridizes to the a target sequence present in said amplicon amplified target sequence under stringent conditions to form a probe:target hybrid stable for detection, wherein said oligonucleotide the base sequence of said target binding region has a base sequence which is 100% fully complementary to the base sequence of said amplified target sequence, wherein the said

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

amplified target sequence is being selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, wherein said probe does not comprise a base region in addition to said target binding region that is capable of stably binding to said amplicon under said conditions, and wherein said probe does not hybridize to nucleic acid derived from a non-Cryptosporidium organism in the said test sample to form a probe:non-target hybrid stable for detection under the stringent said conditions.

123. (Currently Amended) The method of claim 119 further comprising a second amplification primer oligonucleotide, said second primer comprising an oligonucleotide a target binding region, wherein the base sequence of said target binding region having a base sequence which is 100% fully complementary to a the base sequence of a target sequence present in target nucleic acid derived from Cryptosporidium organisms, said target sequence being selected from the group consisting of SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58 and SEQ ID NO:64.

wherein said target binding region hybridizes to said target sequence under said conditions.

wherein said second amplification oligonucleotide does not comprise a base region in addition to said target binding region that is capable of stably binding to said target nucleic acid under said conditions, and

wherein said second amplification oligonucleotide optionally includes a 5' sequence that is recognized by an RNA polymerase or that enhances initiation or elongation by an RNA polymerase.

124. (Currently Amended) The method of claim 123 further comprising the step of determining the presence of the amplicon amplified target sequence in a said test sample with a hybridization assay probe, wherein said probe comprises an oligonucleotide which a target binding region that hybridizes to the a target sequence present in said amplicon amplified target sequence under stringent conditions to form a probe target hybrid stable for detection, wherein the base

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

sequence of said oligonucleotide target binding region has a base sequence which is 100% fully complementary to the base sequence of said amplified target sequence, wherein the said amplified target sequence is being selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, wherein said probe does not comprise a base region in addition to said target binding region that is capable of stably binding to said amplicon under said conditions, and wherein said probe does not hybridize to nucleic acid derived from a non-Cryptosporidium organism in the said test sample to form a probe:non-target hybrid stable for detection under the stringent said conditions.

Claims 125-129 (Canceled)

- determining the presence of the amplicon amplified target sequence in a said test sample with an oligonucleotide probe, wherein the base sequence of said probe is fully complementary to the base sequence of the a amplified target sequence present in said amplicon, and wherein the said amplified target sequence is being selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4.
- 131. (Currently Amended) The method of claim 73 further comprising a second amplification primer oligonucleotide, comprising a target binding region, wherein the base sequence of said second primer target binding region is fully complementary to a the base sequence of a target sequence present in target nucleic acid derived from *Cryptosporidium* organisms, said target sequence being selected from the group consisting of SEQ ID NO:45, SEQ ID NO:51, SEQ ID NO:57 and SEQ ID NO:63.

wherein said target binding region hybridizes to said target sequence under said conditions.

Page 25 of 37

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

wherein said second amplification oligonucleotide does not comprise a base region in addition to said target binding region that is capable of stably binding to said target nucleic acid under said conditions, and

wherein said second amplification oligonucleotide optionally includes a 5' sequence that is recognized by an RNA polymerase or that enhances initiation or elongation by an RNA polymerase.

- of determining the presence of the <u>amplicon</u> amplified target sequence in a <u>said</u> test sample with an oligonucleotide probe, wherein the base sequence of said probe is fully complementary to the base sequence of the <u>amplified</u> target sequence <u>present in said amplicon</u>, and wherein the <u>said amplified</u> target sequence is being selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4.
- 133. (Currently Amended) The method of claim 73 further comprising a second amplification primer oligonucleotide; comprising a target binding region, wherein the base sequence of said second primer target binding region is fully complementary to a the base sequence of a target sequence present in target nucleic acid derived from *Cryptosporidium* organisms, said target sequence being selected from the group consisting of SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58 and SEQ ID NO:64,

wherein said target binding region hybridizes to said target sequence under said conditions,

wherein said second amplification oligonucleotide does not comprise a base region in addition to said target binding region that is capable of stably binding to said target nucleic acid under said conditions, and

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

wherein said second amplification oligonucleotide optionally includes a 5' sequence that is recognized by an RNA polymerase or that enhances initiation or elongation by an RNA polymerase.

- of determining the presence of the <u>amplicon</u> amplified target sequence in a <u>said</u> test sample with an oligonucleotide probe, wherein the base sequence of said probe is fully complementary to the base sequence of the <u>amplified</u> target sequence <u>present in said amplified</u>, and wherein the <u>said amplified</u> target sequence is <u>being</u> selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4.
- 135. (Currently Amended) The kit of claim 74, wherein each of said oligonucleotides has a base region which the base sequence of said target binding region of each said oligonucleotide is at least 80% complementary to the base sequence of the said target sequence of said oligonucleotide.
- 136. (Currently Amended) The kit of claim 74, wherein each of said oligonucleotides has a base region which is 100% the base sequence of said target binding region of each said oligonucleotide is fully complementary to the base sequence of the said target sequence of said oligonucleotide.
- 137. (Currently Amended) The kit of claim 74, wherein the base sequence of each of said oligonucleotides oligonucleotide is at least 80% complementary to the base sequence of the said target sequence of said oligonucleotide.

- 138. (Currently Amended) The kit of claim 74, wherein the base sequence of each said oligonucleotides oligonucleotide is fully complementary to the base sequence of the said target sequence of said oligonucleotide.
- 139. (Currently Amended) The kit of claim 83, wherein each of said oligonucleotides has a base region which the base sequence of said target binding region of each said oligonucleotide is at least 80% complementary to the base sequence of the said target sequence of said oligonucleotide.
- 140. (Currently Amended) The kit of claim 83, wherein each of said oligonucleotides has a base region which is 100% the base sequence of said target binding region of each said oligonucleotide is fully complementary to the base sequence of the said target sequence of said oligonucleotide.
- 141. (Currently Amended) The kit of claim 83, wherein the base sequence of each of said oligonucleotides oligonucleotide is at least 80% complementary to the base sequence of the said target sequence of said oligonucleotide.
- 142. (Currently Amended) The kit of claim 83, wherein the base sequence of each of said oligonucleotides oligonucleotide is fully complementary to the base sequence of the said target sequence of said oligonucleotide.
- 143. (Currently Amended) The kit of claim 85, wherein each of said oligonucleotides has a base region which the base sequence of said target binding region of each said oligonucleotide is at least 80% complementary to the base sequence of the said target sequence of said oligonucleotide.

- 144. (Currently Amended) The kit of claim 85, wherein each of said oligonucleotides has a base region which is 100% the base sequence of said target binding region of each said oligonucleotide is fully complementary to the base sequence of the said target sequence of said oligonucleotide.
- 145. (Currently Amended) The kit of claim 85, wherein the base sequence of each of said oligonucleotides oligonucleotide is at least 80% complementary to the base sequence of the said target sequence of said oligonucleotide.
- 146. (Currently Amended) The kit of claim 85, wherein the base sequence of each of said oligonucleotides oligonucleotide is fully complementary to the base sequence of the said target sequence of said oligonucleotide.
- 147. (Currently Amended) The kit of claim 88, wherein each of said oligonucleotides has a base region which the base sequence of said target binding region of each said oligonucleotide is at least 80% complementary to the base sequence of the said target sequence of said oligonucleotide.
- 148. (Currently Amended) The kit of claim 88, wherein each of said oligonucleotides has a base region which is 100% the base sequence of said target binding region of each said oligonucleotide is fully complementary to the base sequence of the said target sequence of said oligonucleotide.
- 149. (Currently Amended) The kit of claim 88, wherein the base sequence of each of said oligonucleotides oligonucleotide is at least 80% complementary to the base sequence of the said target sequence of said oligonucleotide.

- 150. (Currently Amended) The kit of claim 88, wherein the base sequence of each of said oligonucleotides oligonucleotide is fully complementary to the base sequence of the said target sequence of said oligonucleotide.
- oligonucleotide, wherein said third oligonucleotide has an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in from 18 to 35 bases in length that hybridizes to a target sequence contained present in target nucleic acid derived from a Cryptosporidium organism organisms under said conditions, and wherein the said target sequence being of said third oligonucleotide is selected from the group consisting of SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26 and SEQ ID NO:28.
- 152. (Currently Amended) The kit of claim 151, wherein each of said oligonucleotides has a base region which the base sequence of said target binding region of each said oligonucleotide is at least 80% complementary to the base sequence of the said target sequence of said oligonucleotide.
- 153. (Currently Amended) The kit of claim 151, wherein each of said oligonucleotides has a base region which is 100% the base sequence of said target binding region of each said oligonucleotide is fully complementary to the base sequence of the said target sequence of said oligonucleotide.
- 154. (Currently Amended) The kit of claim 151, wherein the base sequence of each of said oligonucleotides oligonucleotide is at least 80% complementary to the base sequence of the said target sequence of said oligonucleotide.

Serial No. 09/954,695 Atty. Docket No. GP116-02.UT

155. (Currently Amended) The kit of claim 151, wherein the base sequence of each of said oligonucleotides oligonucleotide is fully complementary to the base sequence of the said target sequence of said oligonucleotide.